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Article Title: Canadian COVID-19 population serological survey utilizing antenatal serum samples: a retrospective seroprevalence study

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Peer review comments and author response

REVIEWER 1: Dr. Pete Driezen, University of Waterloo

Using antenatal serum samples collected during 2020, the authors estimated the seroprevalence of SARS-CoV-2 infection in Canada during 3 time periods in 2020 prior to mass vaccination. Results from the study demonstrate that seropositivity rates in Canada were 1.5 to 10 times greater than documented PCR positive rates, indicating public health tracking systems were under-reporting SARS-CoV-2 infections. Importantly, these data document seropositive cases of infection in February 2020 prior to official declaration of COVID-19 as a pandemic in March 2020 by the WHO. Findings are consistent with other studies that indicate SARS-CoV-2 infection was more prevalent that suggested by PCR testing alone. This is a well-written, and important study, documenting the magnitude of SARS-CoV-2 infection in Canada using routinely collected data. That said, I have some questions and suggestions that are intended to strengthen the reporting of this study.

Major concerns:

1. Page 2, line 18: Clarify what is meant by "variable testing capacity" -- i.e., be more specific so the term is clear to readers who might be less familiar with such terms.

Limited by word count- not expanded upon given references provided demonstrate this.

2. Page 2, lines 34-36: "With a very high uptake among all pregnant persons ranging from 93-96% in Canada (13-15), these sera are highly representative of the greater population of reproductive age..." Is this generally true across all provinces, or does this differ provincially? Especially since health care falls under the purview of provincial governments, not the federal government.

This refers to the uptake of all pregnant persons to having antenatal sera taken from prior studies, this is not from public health data for direct comparison.

3. Page 3, line 16: "restrictions" -- perhaps clarify what these restrictions were or give some examples of COVID-19 mitigation measures that were introduced during this time period.

Limited by word count- felt non-contributory given this is not an analysis on efficacy of preventative measures or assessment of regulations. We hope to see this research incorporated into reviews on this in the future.

4. Page 3, line 27: "Pregnant persons" -- I understand that the authors are using inclusive language here (and in other spots in the text, but does that really make sense in this case? I.e., "pregnant persons" and "pregnant people" are less specific terms than "pregnant females" which would be the case (biologically) and clearly states the target group upon which the study is based. Suggest replacing mentions of "pregnant persons" and "pregnant people" with the more specific term "pregnant females". In addition, this makes the terminology consistent throughout (i.e., when data from Statistics Canada are referred to, the population of interest in those cases is "females", i.e., page 4, first paragraph).

(Ed note: It is CMAJ Group style to use pregnant persons).

5. Page 3: "Given the relatively continuous flow of samples from pregnant persons, selection of all eligible samples (e.g. adequate volume and integrity) from a set time period was conducted to provide proportionate sampling based on population size in each jurisdiction" -- In either the results section or as a supplementary table, it would be useful to provide a table showing the total samples available in each province (where samples were available) and the total eligible.

Included in text of results section for each time period and province. Information not available as total eligible as no provincial record available regarding all pregnant person at any given time to reference. 'Adequate volume and integrity' were the only eligibility requirements and explanation of how many samples did not meet this criteria not felt to be contributory. Available for public health reports if required.

6. Page 3, line 44: "96%-63%" -- I recognize the authors are reporting sensitivity of the assays used here (and so are reporting high to low sensitivity), but I think phrasing this as "63%-96% is a more natural way to do so (i.e., reads a little more naturally).

Adjusted

7. Page 4, first paragraph. Regarding the direct standardization of seroprevalence -- Age-standardization within province allows for comparisons WITHIN provinces over the three time periods. HOWEVER, data presented in Fig 1 for period C suggests that comparisons are BETWEEN provinces. If this is what is intended, would it be better to standardize to the Canadian population of females (at least from these 10 provinces) (or 8 provinces, excluding AB and PE)? This allows for more meaningful comparisons across provinces.

Results adjusted- raw seroprevalence only reported now. See updated Figure 1 and 3.

Also, in Fig 1, why is the confidence interval for the age-adjusted rate so large for ON and QC? This seems odd given the CIs for adjusted rates in the other provinces are not much different from the unadjusted rates in those provinces. Especially when the total # of antenatal samples is 6114 in ON in Period C? (I wouldn't have expected a wider CI in this instance).

Figure 1 and Table 1 revised.
Re the wider CI's for age adjusted rate for ON and QC in Table 1, this reflects the wider difference in seroprevalence rates between the age groups. Each group had high numbers contributing to a stronger impact on the CI than in other provinces where the youngest and oldest age groups had small numbers contributing less to the CI limits.

8. Page 4: "PCR Confirmed Cases Comparison" -- Why are the territories included in the groupings here, when data on antenatal samples only came from the provinces? Adding them in seems odd -- suggest re-running that analysis using only the provinces for the groupings and excluding the 2 territories...

This resulted from the StatCan grouping and was not by our selection for this data. This is how the provinces and territories are grouped for PCR results based on health service connections and we are then required to compare against these groupings for the individual provinces. Unable to exclude the territories for this reason.

9. Page 4, lines 34-36: "The number of births in 2020 was used as a proxy for the number of pregnant people in each province adjusted for the relevant time period" -- "adjusted for the relevant time period" is somewhat unclear. Please clarify.

The adjustment was taken as the number of births that would have occurred in the time period under investigation, given that the number for 2020 was over 366 days. For example, if the time period was to December 6th 2020, that would be the 341st day. 341/366 (leap year) = 0.93, so the number of births would have been adjusted as the total * 0.93 or 93% of the total.

10. Page 4, lines 45-48: "Rate ratios and confidence intervals of seroprevalence to PCR rates were calculated using a Chi-square approximation" -- provide more details about how this test was conducted. Calculated in R? Use the base packages or as part of an additional library/package?

See line in Statistical Analysis section: "All analyses were carried out in R version 4.1.1 (2021-08-10) (21)."

11. Page 5, line 15: "Sampling period A was selected to determine if there was any notable SARS-CoV-2 circulating within the general population prior to declaration of the COVID-19 pandemic" -- please provide the date of declaration of COVID-19 as a pandemic in Canada -- do you mean the date it was officially declared as a concern in Canada or the date the WHO declared COVID a pandemic?

Yes- the date it was declared as a pandemic by WHO as per reference. Media reports available reporting individual travel related cases during this period, but not formally documented in the literature hence the value of this research in demonstrating community spread during this period eg https://news.gov.bc.ca/releases/2020HLTH0052- 000356

12. In that same paragraph, "A total of 7329 antenatal serum samples" -- please provide more details for each period in a table (see comment #5 above). I.e., List the total number of antenatal samples in each province along with total that could not be used, and antenatal "participation" rate for the province, or what percentage of pregnancies have an antenatal sample stored. This is important information because it provides insight into differences by province that could affect interpretation of results. Also provide median age, interquartile range, and range. This could be a supplementary table only, I think.

As above- the only information regarding uptake of antenatal participation is provided in the references referred to with the original statement. This data is not recorded nationally or provincially and was not part of the ethics agreement for linkage with the samples provided- in other words, it would not have been possible to identify the individuals by name to determine if all known pregnancies in the province at the time were included. Number of samples is listed in text of document. Antenatal participation rate is not available information from national data reporting. Appreciate reviewer's interest but felt not to be contributory to this manuscript.

13. Page 6, line 41: "The seroprevalence was on average 4.3 times higher than the PCR positivite rate for

the ten provinces." (a) How was this computed? It's not really clear based on the results presented. (b) Correct the spelling for "positive" -- currently, it reads "positivite".

This is an average of the seroprevalences reported, a crude calculation for the purposes of brevity in this section. New addition of median result with CI's included
Spelling corrected.

14. Page 7, Interpretation, line 13: "Despite this, the age-adjusted seroprevalence of 0.03% in BC, 0.19% in SK and raw rate of 0.35% in AB, demonstrate early spread" -- I think it's important to qualify this early spread as "early, but generally low, community spread"

Adjusted

15. Page 7, lines 22-24: "The evolution of the pandemic between provinces and within each province has shown distinctive differences likely due to varying public health policy and population density" -- suggest replacing the word "likely" with "possibly". No data are presented about public health policy or testing the effects of public health policies. In other words, this interpretation ("likely due to varying public health policy") is more speculative and not supported by any data present.

Adjusted

16. Page 7, line 34: "CBS samples from November 2020 demonstrated a lower seroprevalence in ON" -- clarify whether data from Canadian Blood Services are based on all donors or female donors only. Qualifying what is being compared helps readers better understand the comparison made and possible limitations of that comparison.

We did not specify female donors hence presume readers will know that this is referring to the study cohort referenced, not a sub- analysis.

17. Page 7, line 38: "These discrepancies may reflect the selection bias of healthy blood donors" it would helpful to also point out that the age range of CBS donors differs from the age range used in your study -- min age for donation is 17 with no upper age limit.

Limited by word count to expand further on this section.

18. Page 9, lines 23-25: "Antenatal serosamples represent a highly valuable window into the population health burden of this pandemic and other infectious diseases of public health signficance." (a) Significance is incorrectly spelled. (b) Add a qualifier, i.e., "the population health burden of this pandemic and possibly other infectious diseases".

Adjusted

19. Page 22, Figure 2 -- Suggest enlarging the graph; it is a bit squished. Also could include point estimate directly on each plot (or in a supplementary table). This way, readers have full access to the point (and interval) estimates,

Adjusted

20. Page 23, Figure 3: Again, how are the rates standardized? What is the standard population? Methods seem to suggest it is province specific, but here trends suggest comparisons between provinces, so wouldn't it be better to standardize to the group of provinces used for the figure? That way, it's possible to compare the trends more directly... While this makes it difficult to compare rates across figures (and indeed estimates will differ because different standard populations are used), with respect to each figure itself (1 and 3), it is possible and meaningful to compare rates and trends across provinces.

Adjusted to present only raw seroprevalence results. Figure 1 & 3 adjusted, table 1 updated.

21. Page 24, Figure 4. Please specify whether the data are for Period C only or whether data for all study periods (A, B, and C) are presented. If the figure presents data combined for all periods, perhaps present small multiples for each period so it's possible to see any changes by province through time. The reason would be that the time and location of seropositive cases may differ from the time and location of seronegative, possible in more rural/northern parts of BC, SK,, ON, and QC.

Adjusted. Map per period not deemed to add to value of manuscript given smaller numbers of participating provinces for other time periods.

Also, the distributions follow population density in each province (generally speaking), so another reason to split by time. Figure may have limited utility this way, so suggest move it to a supplementary figure with small multiples for each time period (i.e., 1 map per period).

22. Page 27, Supplementary Table: Footnote the definitions of "anti-S" and "anti-N", where Anti-S = anti-spike protein assay; Anti-N = anti-nucleocapsid protein assay. IgG, IgA, and IgM might be more familiar, but also good to include definitions in footnote, i.e., immunoglobulin G, A, and M.

This is included in the reference (18) – new line added indicating specificity 100% (95%CI 99.1-

100%)

Supplementary table 1 updated

The table should also list sensitivity and specificity information for each unique assay -- this is important for interpreting the results. If sensitivity/specificity for the different tests vary substantially, how might this influence the findings? Include as a limitation?

Minor comments:

1. Page 3, line 7: Is there really any need to list the provinces, given the article is in CMAJ Open? I could understand if not CMAJ. This, however, is a minor quibble; for brevity, could avoid listing...

Retained given feel this is useful for international readership and to allow for abbreviations to be delineated for the remainder of the manuscript.

2. Page 6 -- the section header "Raw and Age-Adjusted Seropositivity by Province for Time Period C" could be removed, I think, so that this section overall is consistent with the previous sections.

Removed as per reviewer's suggestion

3. Page 7, line 45: maybe replace "cultures" with "cultures and ethnicities"?

Adjusted

REVIEWER 2: Dr. Dena Schanzer, Public Health Agency of Canada

In this study, the authors report on the results of a retrospective serological surveillance study that utilized residual blood samples from prenatal blood testing across Canada to estimate the seropositivity for SARS-CoV-2. The main results cover specimens taken during a three-week period in November 2020 (period C), when data from all 10 provinces was available, and approximately 1% of the Canadian population had had a PCR-confirmed infection. Vaccination was approved in late December, starting with the most vulnerable populations (residents of long-term care homes, at-risk health care workers, and residents of remote locations).

The objective is stated as estimating seroprevalence for Canadian females of reproductive age at three points in time prior to the initiation of vaccination. A secondary objective of comparing the seroprevalence with surveillance rates based on PCR-confirmed cases at the regional level is identified in the methods section.

General Comments:

My main concern is that neither seroprevalence nor the rate ratio (seroprevalence/PCR-confirmed cumulative case rate) among pregnant women are generalizable to the broader population of Canadian women aged 20-49. The data provided in Table 1 seems to contradict this assumption with PCR-confirmed cumulative case rates for period C about 2-fold higher in women aged 20-49 compared to pregnant women. I would imagine that pregnant women would be more health conscious and hence less likely to visit high risk venues and more likely to get tested on advice of their doctor. Or, would routine testing of patient-facing health-care workers resulting in a higher testing rate among their same-age peers?

The generalizability of the seroprevalence estimates to the broader population of all reproductive age (people or women) has not been established in the manuscript.

Technically, this is not just a retrospective serological surveillance study, but requires a mathematic modelling component to extrapolate the seroprevalence estimated for pregnant women to all Canadian women aged 20-49. Reporting guidelines (both the one included in this submission as well as those from the Equator network) require a clear statement of the study design. For the extrapolation component, reporting guidelines require a full list of assumptions. Pregnant women are likely to be more health conscious than their same-age peer, making efforts to avoid high risk exposures, and to seek medical advice, including testing if sick, or a close contact of a confirmed case. There is a noted difference in the PCR-confirmed rates for pregnant women and women aged 20-49 presented in Table 1, suggesting pregnant women are not representative of their same-age non-pregnant peers. As peer reporting guidelines, a review of the relevant literature may provide information of parameter values/ assumptions required to account for these differences.

Presenting the study results as two components seems to me to be the easiest solution. I strongly recommend that the authors provide the results of the serological survey for pregnant women as the main objective, especially the overall estimates for the Canadian population of pregnant women, along with the

95%CI. Next, assumptions used to extrapolate to the broader population must be clearly stated. References should be provided where published parameter values exist. As with any modelling study, confidence intervals would not be appropriate for the estimates calculated for the broader population, as uncertainty associated with different risks of exposure and different likelihoods of testing once exposed is likely unknown. There are two obvious naive assumptions: the seroprevalence is the same for pregnant women and their same-age peers; or the likelihood of PCR confirmation among those infected is the same for pregnant women and their same-age peers. These assumptions produce rather different results, and neither many be appropriate. Based on data presented for Ontario, and using the two naive assumptions, seroprevalence for women aged 20-49 could be either 5.7 or 10. I would guess the variation due to assumptions would be similar for the national estimate. A literature search is required to explore plausible ranges for these assumptions. Typically, the results are reported as a sensitivity analysis, as confidence intervals are not available for mathematically modeling studies.

My general impression of the manuscript is that putting together a seroprevalence study has been rather challenging. While there was considerable interest by both provincial and federal public health agencies for estimates of immunity during 2020, the utility of this study now is more likely oriented towards pandemic planning for future preparedness, or possibly assessing the value of a serological study to aid in assessing the population level immunity towards the next wave/variant. The goals of the study should be reviewed in light of the current context, but also because they are not well aligned with the results presented. The results, as presented (Table 1 for example) focus on provincial estimates, while the stated objective says the study aims to estimate seroprevalence for Canadians of reproductive age. My impression is that sample sizes are too small to compare provincial estimates, and national estimates are not included in Table 1. I am left wondering if the provincial variation in seroprevalence simply reflects the provincial variation documented by the PCR-confirmation rates?

Points of clarification:

1) By the way, PCR-positivity rates would typically refer to the rate per specimen tested, rather than a rate of cases per population. Perhaps, this could be labeled as PCR-confirmed rates.

Adjusted- changed to prevalences as per Editor's suggestion.

2) What population were the rates standardized to? This is not stated. I'd assume you did a direct standardization to the population of females aged 20-49 in order to compare the seroprevalence rate to the PCR-confirmed rate for females age 20-49 in Table 1. Do you not want to compare the seroprevalence rate, without any age adjustment to the PCR-confirmed rate for pregnant persons? If you are using direct standardization, I'd expect to see both the unadjusted and adjusted seroprevalence rates in Table 1. Direct standardization inflates the standard error significantly (figure 1). Given the lack of generalizability to the broader population, the presentation of the direct standardization should be reconsidered.

Adjusted and Table 1 updated.
This ("creating a weighted average of them, where the weights are the proportions in each age group in the standard population.") is how the age- standardization was done, as this is the generally accepted method. We are hopeful that our citations of the methods (delta method for confidence intervals) and R package used (dsrTest) are sufficient for other researchers to satisfy themselves with the appropriateness of our methodology. It also allows for others to choose to use the same methods if they desire to do so. As this is not specifically a manuscript about prevalence estimation methods, and we are constrained for space, further explanation seemed unnecessary.

3) What do you mean by generalizability to the broader population? My initial impression was where did this come from? From Table 1, PCR-confirmed rates were considerably higher (approximately a 2-fold difference) in all provinces for women aged 20-49 than for pregnant women. I would imagine that pregnant women would be less likely to visit high risk venues and more likely to get tested on advice of their doctor. Given the large difference in the PCR-confirmation rates, is the comparison with rates for the population of women aged 20-49 meaningful?

Table 1 comparisons are between StatsCan results and a separate surveillance study CANCOVID-Preg which relies on pregnancy care provider reporting of PCR positive cases which its own limitations in terms of capturing pregnant patients despite best efforts to make this a national reporting program. We feel that this Table demonstrates that serosurveillance captures prevalence more accurately than two other programs reliant on PCR reporting at that time.

The majority of these antenatal samples are collected at the first appointment along with the diagnosis of pregnancy- usually between 5-10 weeks of gestation. This is not a reflection of the number of pregnancies that were continued or planned. Rates of unintended/un planned pregnancies for Canada are sparse and outdated, largely from survey data, but to presume that these samples are from patients who are planning, preparing and adjusting their activities for a wanted pregnancy, is in itself an assumption. This study: Kelly JC, Raghuraman N et al. Preprocedural asymptomatic coronavirus disease 2019 cases in obstetrical and surgicalunits.AmJObstetGynecol. 2021; 224: 114-116 Demonstrated a 15 x higher rate of asymptomatic obstetric than surgical patients- suggests that obstetric patients do not have lower rates of infection.

4) I don't see the results associated with the stated objectives. Estimates of seroprevalence for Canada should be reported in the results section of the abstract as this is the stated objective. Given the data presented, I'd consider the seroprevalence for pregnant women in Canada for period C and the corresponding rate ratio (seroprevalence/ reported PCR-confirmation rate) for pregnant women to be the main results. Both point estimates and the 95%CI should be reported in the results section of the abstract. As per general comments, above, the results for the seroprevalence survey should be reported separate from the extrapolation to the broader group, along with the main assumptions used in the extrapolation. Extrapolated results should not have confidence intervals, the uncertainty associated with different risks of exposure and different likelihoods of testing once exposed is unknown. A sensitivity analysis should be included.

Abstract adjusted and new results included.

5) Seroprevalence estimates don't seem to be available for 3 waves, nor for the 3 periods. I'd suggest restating the study objective to agree with the available results as presented. From the Canadian PCR-confirmed surveillance data, it appears that the third wave ended in the summer of 2021. I'd suggest calling these periods rather than waves. In addition to reporting the seroprevalence estimates and the rate ratios in Table 1, I'd suggest report the summary results for period A and B for all provinces combined in this Table as well.

Adjusted- now reads as periods as opposed to waves.
Supplementary table 2 provided.

6) The overall seropositivity rate (<6%) reported in the interpretation section of the abstract should be reported in the results section of both the abstract and main document, along with 95%CI, for statistical estimates. The discussion/ interpretation sections should not introduce new quantitative results. These should be reported in the appropriate results section and most likely in tables.

Adjusted- sentence deleted. Discussion/ interpretation adjusted to not introduce new results.

7) To support the conclusion (During the time periods sampled, public health tracking systems in all provinces were underreporting infections by four-fold on average) the rate ratios should be reported with 95%CI for each of the 3 periods (for the study population), or the statement reworded. An estimate of precision is not available for the seroprevalence estimate for the broader population, though results from a sensitivity analysis are used instead.

Adjusted (removed)

8) While it is well established from surveillance data of PCR-confirmed cases provided by PHAC and the provinces that the level of viral activity varied significantly by province and regions and within provinces and over time, what is not clear is whether this study generally confirms the differences identified by PCR confirmation or highlights important differences. Comments on important provincial differences need further clarification. In other words, are any of these provincial differences important enough that larger seroprevalence surveys should be incorporated to the general surveillance plan during a pandemic?

See cover letter

9) The abstract states that on average, the seroprevalence estimates suggest that cumulative incidence was 4-fold higher than would be suggested by the cumulative number of PCR-confirmed cases. The statement "The seropositivity rates were 1.6 to 10-fold higher than the documented concurrent PCR positive rates in these jurisdictions" should be moved to results section and clarified or removed. Is this the range in provincial rate ratios? I am wondering if the differences in the provincial rate ratios are statistically significant overall (after accounting for multiple comparisons). The point estimate of 4 and range of 1.6 to 10 suggests a rather imprecise estimate which does not appear to be the case for the larger provinces.

Statement adjusted to include specific rate-ratio range of statistically significant rate ratios. Higher rate ratios are found for all provinces where direct comparison of age adjusted seroprevalence and PCR prevalence can be made (BC, ON, and QC). For provinces where seroprevalence is compared to regional PCR prevalence, rate ratios vary widely and fewer were statistically significant.

Specific comments:

1) Abstract: The statement "Presence of seropositivity as early as February in all jurisdictions reflects the extent of SARS-CoV-2 transmission in the early phases, prior to pandemic declaration." is an interpretation. It would help to provide references to the baseline seropositivity, perhaps from earlier blood sample (2019 or earlier) to get an idea of false positive rate and interpretation of results from Period A.

Sentence adjusted

2) The estimate of 6% positivity should be qualified with a date as you mention three periods.

Adjusted- 'at end the end of 2020' indicative of time period C.

3) Introduction: Should also mention that the criteria for access to testing (In period A, testing was limited to recent travel to high-risk countries such as China and Italy, or contact with an confirmed case) severely limited access to testing in Period A.

Agree- limited by word count- added to Methods section as per below suggestion

4) Methods: Period A, corresponded to very limited lab testing for SARS-CoV-2, and most cases were initially among travelers from high-risk countries. This would create additional implications for generalizability, considering as well that pregnant women would be less likely to travel, especially towards the end of their pregnancy.

Adjusted
Antenatal serology samples are collected at the start of pregnancy- usually at the time of first visit on identification of pregnancy. See above discussion regarding assumptions made in terms of pregnant persons activities, pregnancy planning and continuation.

5) Period C corresponds to the start of second wave (not the second wave).

Adjusted to read as start of the second period of infection

6) Clarification is needed on how the number of births in 2020 was used as a proxy for the number of pregnant people. How long is one considered pregnant for? Perhaps you assumed women realize they

are pregnant on average 1.5 months into the pregnancy, then I figure that each 100 births in 2020 would account for approx 62 pregnant females for each of the 3 periods? What did you assume?

7) A reference to the Chi-squared approximation for calculating 95% CI for rate ratios is needed. I am not familiar with this approximation, so I've provided a link to the formula for calculating the 95%CI for rate ratios and odds ratios that I am more familiar with:

a. with an online calculator: https://www.medcalc.org/calc/relative_risk.php

b. https://influentialpoints.com/Training/confidence_intervals_of_risk_ratio_odds_ratio_and_rate_ratio-principles-properties-assumptions.htm

See line in Statistical Analysis section: "All analyses were carried out in R version 4.1.1 (2021-08-10) (21)."

8) Results: A reference to the table where the full results are presented (for Period A, B, C) should be provided. A table layout makes comparisons easier. Only the more important results should be highlighted in the text.

Provided in supplementary table 2

9) I suggest providing the overall (BC+AB+SK) seroprevalence rate with 95%CI for period A from the study population.

Limited by word count, esp given new additions.

10) Please clarify the statement "No formal reporting of PCR-positive cases occurred during this sampling period. ", as PCR-confirmed cases were reported for Canada, suggest a prevalence of 0.0006%. A hundred-fold difference wouldn't surprise me.

Adjusted to say pregnant persons

11) The early federal testing criteria were very restrictive and there did not seem to be any testing strategy in place to detect community transmission in a timely manor. Pregnant women is not the best group to test due to lower risk of exposure. Evidence of community transmission seemed to emerge from testing hospitalized patients with an otherwise unidentifiable viral pneumonia. Can you discuss viable strategies to detect community transmission earlier?

Limited by word count, see cover letter.

12) Could you include additional decimal digits in the 95%CI? I'd suggest replacing the lower CI of 0.00 with at least 2 significant digits. The lower CI is not a hard zero and is often used when developing public health policy. When the prevalence is low, rather than reporting percentages, ie, per 100 population, denominators of 1,000 to one million could be used.

Adjusted

13) On the topic of calculating 95%CI for OR or RRs when one of the cell counts is 0 (as in Table 1, PEI and NL), the estimate is actually calculable. The RR is zero, as is the lower CI. The upper CI is typically calculated by using continuity corrections, as the usual formula for the standard error is only an approximation. The calculator mentioned above by Medcalc (https://www.medcalc.org/calc/relative_risk.php) includes a correction for zero cells. An article by Moller and Ahrenfeldt (https://www.mdpi.com/1660-4601/18/11/5527/htm), illustrates a number of other options; basically, by using exact measures or a continuity correction. Not sure if this is needed, as you could report the results at the regional level only rather than including provincial results with the zero cells. The statement that "NL tested 33 serological samples for this period and detected no confirmed seropositive samples" should state the estimate of seroprevalence as 0.0% (95% CI: 0.0 -10.6). (A binomial calculator is available at https://sample-size.net/confidence-interval-proportion/ . This calculator provides a one-sided 97.5% confidence interval using an exact calculation for zero cells).

Updated table 1

14) Interpretation: Regarding the statement "During the second wave of infection (sampling period C), seroprevalence varied widely across provinces, from 0.24% in NL to 5.95% in QC.": Please comment on whether the provincial variation in seroprevalence rates is statistically significant, and whether these differences are consistent with the differences in the PCR-confirmation rates in pregnant women. Do the rate ratios, indicating the proportion of infections confirmed by each province, vary?

Limited by word count given new additions and results.
Table 1 and supplementary table 2 included to address aspects of this.
Revised table 1 to include raw seroprevalence for comparison purposes.

15) Re: "Our findings also demonstrate important differences when compared to positive SARS-CoV-2 PCR test rates for the same time periods, with seropositivity rates found to be consistently higher. This is reflected in the results seen in BC and MB for time period C." Why are the differences in BC and MB more important? It is not obvious the overall differences in the estimated rate ratios (for the study population) by province are statistically significant (after account for multiple comparisons).

Paragraph deleted due to word count requirements

16) I'd suggest limiting the repetition of the study results in the interpretation section. Most seroprevalence studies have found that seroprevalence rates are higher than PCR-confirmation rates. I'd suggest limiting the discussion primarily to your overall results. If you wish to highlight provincial differences additional discussion of the importance of implication of these differences should be included. In the sub-section on comparison with other studies, I'd like to see additional discussion of a comparison of your overall rate ratio for Canada (seroprevalence/PCR-confirmation rates for pregnant women) with other studies.

Adjusted- section deleted.
Additional discussion limited and comparison with meta-analysis results discussed in introduction and not felt to be worthwhile to re- discuss in interpretation section, as reviewer has noted previously, the finding of seroprevalence exceeding PCR rates is well documented.

17) Re: "This study presents one of the largest serosurveillance studies performed to date on a representative sample of Canadian reproductive age adults": The claim that this is a representative sample of Canadian reproductive age adults (or females) has not been substantiated. See comments related to reporting on mathematical modelling studies/extrapolation.

Sentence deleted.

18) Re timeliness: I'd suggest further discussion of the feasibility of improving the timeliness and precision of a serosurveillance study, as this could be useful for future Pandemic planning purposes!

See new data demonstrating timeliness capacity at provincial level.

19) Conclusion: Re: "We also document variability in how each province experienced waves of SARS-CoV-2 infection and show that seroprevalence rates were 1.5-10 times greater than reported PCR-positive case rates." I don't follow this statement. Again, what is the range 1.5-10? Why is it so large? I suspect that the sample size is too small in some of the smaller provinces and that is why you have such a large range? Or did some provinces limit testing?

Conclusion rewritten to include single result rather than range.
Reviewer's assumption regarding small sample size (eg 33 samples in NL) is correct regarding the prior statement.

20) Provincial differences in the timing of waves are already well documented. Do you mean that your study confirms the variability in this timing? You only have data for 3 periods. If the rate ratios for period C vary significantly by province, I'd suggest plotting these in a forest plot (as is typical in a meta-analysis) to provide a better visual cue of this variability

REVIEWER 3: Dr. Affan Shoukat, Yale University

Dear Authors,

I have reviewed the manuscript as submitted. In this manuscript, the authors conduct a serological surveillance study to estimate the prevalence of COVID-19 in Canada. They selected three main time periods (all within 2020) and found that infections in Canada were severely under-ascertained. (The underreporting is expected given variable testing supply and mild/asymptomatic infection). I am recommending acceptance, though I do have some minor comments.

The authors claim that their samples (i.e. women of reproductive age) are representative of the Canadian population by geographic location and socioeconomic diversity. This is an interesting claim and does make sense to me to some degree, but it would be great to see some more justification of this claim in the main text.

The majority of these antenatal samples are collected at the first appointment along with the diagnosis of pregnancy- usually between 5-10 weeks of gestation. This is not a reflection of the number of pregnancies that were continued or planned. Rates of unintended/un planned pregnancies for Canada are sparse and outdated, largely from survey data, but to presume that these samples are from patients who are planning, preparing and adjusting their activities for a wanted pregnancy, is in itself an assumption. This study: Kelly JC, Raghuraman N et al. Preprocedural asymptomatic coronavirus disease 2019 cases in obstetrical and surgicalunits.AmJObstetGynecol. 2021; 224: 114-116 Demonstrated a 15 x higher rate of asymptomatic obstetric than surgical patients- suggests that obstetric patients do not have lower rates of infection.

As far as I know, Canadian Blood Services also conduct serological data analysis (up to end of 2021 I believe). Have the authors verified their findings with CBS findings?

See reference to this study in interpretation. We felt that direct comparison of their results and ours was not beneficial for the purposes of this manuscript, but in line with their findings we have determined sero-surveillance as a useful public health tool but maintain that antenatal samples do not carry the same bias as CBS- CBS is restricted by location, ethnicity and socio-demographic and health variables of donors.
Antenatal samples are received regardless of location, health status of the person, intention or continuation of the pregnancy and are publicly funded.

Also, could the CBS results be used in this extend to include more time periods (i.e., 2021 as well). I am not sure why only the pre-vaccine era was selected, so perhaps some explanation could be nice.

Overall, this is a timely manuscript and provides a retrospective look into pandemic progression in Canada. I will recommend for publication.